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Authors

Wang, S
Cheng, G
Dong, J
et al.

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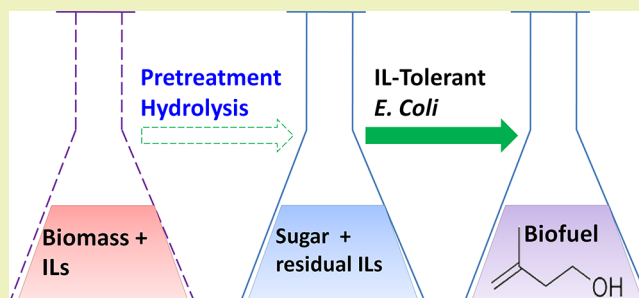
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Tolerance Characterization and Isoprenol Production of Adapted *Escherichia coli* in the Presence of Ionic LiquidsShizeng Wang,[†] Gang Cheng,^{*,†,‡} Jie Dong,^{‡,§} Tian Tian,^{‡,§} Taek Soon Lee,^{‡,§}
Aindrita Mukhopadhyay,^{‡,§} Blake A. Simmons,^{‡,§} Qipeng Yuan,[†] and Steven W. Singer^{*,‡,§}[†]State Key Laboratory of Organic–Inorganic Composites and College of Life Science and Technology, Beijing University of Chemical Technology, North 3rd Ring East, #15, Beijing 100029, China[‡]Joint BioEnergy Institute, 5885 Hollis Street, Emeryville, California 94608, United States[§]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, California 94702, United States

Supporting Information

ABSTRACT: Ionic liquid (IL)-based pretreatment makes lignocellulosic biomass more accessible to enzymes and improves enzymatic digestibility. However, the ILs left in biomass slurry after pretreatment could inhibit activity of enzymes and microbial fermentation. Therefore, it is necessary to develop robust host strains that are IL-tolerant. In this study, we characterized IL tolerance and biofuel production of adapted *Escherichia coli* obtained by adaptive laboratory evolution (ALE). We found that IL-tolerant *E. coli* obtained via ALE by 1-butyl-3-methylimidazolium chloride ($[C_4C_1Im]Cl$) showed improved growth in the presence of four ILs, $[C_4C_1Im]Cl$, 1-ethyl-3-methylimidazolium chloride ($[C_2C_1Im]Cl$), 1-butyl-3-methylimidazolium acetate ($[C_4C_1Im][OAc]$), and 1-ethyl-3-methylimidazolium acetate ($[C_2C_1Im][OAc]$) compared to that of the parent strain. The growth of adapted strain *E. coli* MG1655-A1 was even promoted by $[C_4C_1Im]Cl$ and $[C_2C_1Im]Cl$ of certain concentrations. The adapted strains were further transformed by introducing mevalonate-based metabolic pathway and they showed significantly increased isoprenol titer compared to parent strain *E. coli* MG1655. Furthermore, they were shown to use $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as carbon sources and assimilate the acetate ions. These results indicated that ALE provided promising host strains for one-pot biofuel production.

KEYWORDS: Ionic liquid pretreatment, Adaptive laboratory evolution, Tolerance, Isoprenol, *E. coli*



INTRODUCTION

Using lignocellulosic biomass for production of renewable biofuel and value-added chemicals provides a sustainable and promising solution to the depletion of fossil fuels and environmental pollution.^{1,2} Conversion of lignocellulosic biomass into sugars relies on efficient pretreatment techniques to improve enzymatic digestibility of biomass.^{3–5} Recently, ionic liquid (IL)-based pretreatment processes have emerged and gained much attention for their effectiveness to process a variety of biomasses.^{6–9} Among the ILs investigated for biomass pretreatment, $[C_4C_1Im]Cl$, $[C_4C_1Im][OAc]$, $[C_2C_1Im]Cl$, and $[C_2C_1Im][OAc]$ are the most popular ones because of their remarkable capability to dissolve cellulose.^{10–15} However, a certain amount of ILs would be left in the biomass slurry after pretreatment. ILs' toxicity to cellulases and microbes is one of the major challenges in the application of IL-based biomass pretreatment.⁶ A previous study showed that multiple washing processes were required to remove ILs from biomass.¹⁶ However, extensive washing is not economically feasible on an industrial scale. Therefore, it is of

great importance to develop IL-tolerant microbes that could accommodate high levels of residual ILs (for example, 0.2–5% w/v^{16–18} ILs in hydrolysate) during saccharification and fermentation.^{18–21} In addition, integration of pretreatment, saccharification, and fermentation into one process, known as “one-pot” process,^{22,23} also depends on the development of IL-tolerant strains.

Progress has been made on developing IL-tolerant strains by searching for IL-efflux pumps and IL-assimilating strains from extreme environments, like tropical forest soil and marine environment with high level of salinity and hydrocarbon.^{7,18,23} The mechanism of autoregulation of an IL-efflux pump by *EilR* repressor in *Enterobacter lignolyticus* was revealed by Ruegg et al.¹⁸ The autoregulation system was successfully introduced into *Escherichia coli* to produce bisabolane in the presence of $[C_2C_1Im]Cl$. Frederix et al. found that *ybjj* and *rcdA* were

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involved in IL transportation and the corresponding repressor in a spontaneous IL-tolerant *E. coli* mutant.²² On the basis of this mechanism, *E. coli* DH1rcdA mutant was used as host for one-pot limonene production in the presence of [C₂C₁Im]-[OAc].²² However, the IL-tolerant strains still face some challenges. The plasmids used to express IL transporters would lead to an extra metabolic burden in cells.²⁴ The transporters themselves could also be toxic to cells.¹⁸ The extra selection markers for plasmids would also increase the cost of fermentation. In addition, the application of these IL-tolerant strains was limited by the type and concentrations of ILs.

Adaptive laboratory evolution (ALE) has also been shown to be a useful tool to obtain strains which could tolerate ILs.^{25,26} In our recent study, we obtained adapted strains *E. coli* MG1655-A1 (named as MG 4.7 in ref 27) and *E. coli* MG1655-A2 (named as MG 3.1 in ref 27) via ALE using [C₄C₁Im]Cl.²⁷ Here, we characterized the IL tolerance of *E. coli* MG1655-A1 and *E. coli* MG1655-A2. The adapted strains show increased tolerance to four extensively studied imidazolium-based ILs ([C₄C₁Im]Cl, [C₄C₁Im][OAc], [C₂C₁Im]Cl, and [C₄C₁Im][OAc]) compared to the parent strain *E. coli* MG1655.

The rapid development of C4–C5 alcohols as biofuels has significantly expanded the biofuel contents and could potentially substitute ethanol in future gasoline mixtures.²⁸ 3-Methyl-3-buten-1-ol (isoprenol) is a promising biofuel for its octane numbers and combustion properties.²⁹ Both methylerythritol phosphate pathway and mevalonate pathway can be used to produce isoprenol.^{30,31} To evaluate adapted *E. coli*'s potential in biofuel production, we transformed mevalonate-based metabolic pathway of isoprenol into *E. coli* MG1655-A1, *E. coli* MG1655-A2, and *E. coli* MG1655.³² The transformed strains, named as *E. coli* MG1655-A1-KG1R10, *E. coli* MG1655-A2-KG1R10, and *E. coli* MG1655-KG1R10, showed increased isoprenol yield in the presence of the ILs compared to the parent strain. They could even grow up and produce biofuel when using [C₄C₁Im][OAc] and [C₂C₁Im][OAc] as carbon sources. The adapted *E. coli* showed potential application in reusing and recycling of wastewater containing [C₄C₁Im][OAc] and [C₂C₁Im][OAc] for biofuel production. The development of IL-tolerant *E. coli* by ALE provides a promising host for discovering a novel mechanism of IL tolerance, integrating biofuel fermentation with pretreatment and saccharification by a one-pot process, and recycling wastewater containing ILs.

MATERIALS AND METHODS

Experimental procedures and materials characterization are provided in the Supporting Information.

The strains and plasmids are listed in Table 1.

RESULTS AND DISCUSSION

Increased IL Tolerance and Isoprenol Production of Adapted *E. coli*. *E. coli* MG1655-A1 and *E. coli* MG1655-A2 in this study were obtained via ALE by [C₄C₁Im]Cl. Figure 1 presents growth profiles of the strains without the presence of ILs. The culture volume in 96-well plates was 200 μ L. Both of the adapted strains grew faster and showed greater biomass (*E. coli* cells) yield than *E. coli* MG1655 in 10 h after inoculation (Figure 1). However, they showed less biomass yield after 36 h of cultivation compared to the parent strain. MG1655-A2 and *E. coli* MG1655 showed a stationary phase after the exponential phase. In contrast, *E. coli* MG1655-A1 came into

Table 1. Strains and Plasmid Used in This Study

| strains/plasmids | description | reference or source |
|---------------------------------|--|---------------------|
| Strains | | |
| <i>E. coli</i> MG1655 | parent strain | ATCC 700926 |
| <i>E. coli</i> MG1655-A1 | IL-tolerant strain obtained by ALE | 27 |
| <i>E. coli</i> MG1655-A2 | IL-tolerant strain obtained by ALE | 27 |
| <i>E. coli</i> MG1655-A1-KG1R10 | pJBEI-6829 + JPUB-004507 in <i>E. coli</i> MG1655-A1 | this study |
| <i>E. coli</i> MG1655-A2-KG1R10 | pJBEI-6829 + JPUB-004507 in <i>E. coli</i> MG1655-A2 | this study |
| MG1655-KG1R10 | pJBEI-6829 + JPUB-004507 in <i>E. coli</i> MG1655 | this study |
| Plasmids | | |
| pJBEI-6829 | pBbA5c-MevTsa-MK-PMK | 33 |
| JPUB-004507 | pTrc99A-nudB _{RBS10} -PMD | 32 |

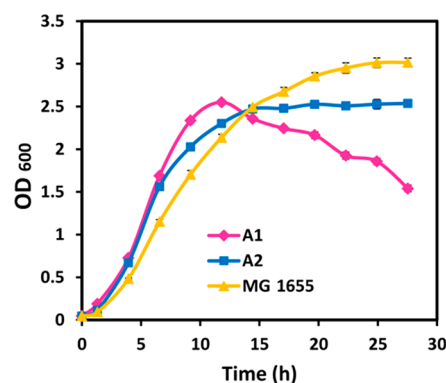


Figure 1. Growth profiles of *E. coli* MG1655-A1, *E. coli* MG1655-A2, and *E. coli* MG1655. The error bars represent standard deviation calculated from each experiment run in triplicate.

the decline phase as soon as the exponential phase ended. It is noted that *E. coli* MG1655-A1 possessed a stationary phase with the ILs in the medium, as shown later in Figures 2 and 3.

The tolerances of *E. coli* MG1655-A1, *E. coli* MG1655-A2, and *E. coli* MG1655 to [C₄C₁Im]Cl, [EMIM]Cl, [C₄C₁Im][OAc], and [C₂C₁Im][OAc] were evaluated by growth assay and IC₅₀. Figure 2 presents growth profiles of them in the presence of [C₄C₁Im]Cl and [C₂C₁Im]Cl. The growth profiles of *E. coli* MG1655-A1 showed a stationary phase which was in contrast to that of *E. coli* MG1655-A1 in the absence of the ILs in the media. When the [C₄C₁Im]Cl concentration was in the range between 50 and 250 mM, the final biomass yield (after 36 h of cultivation) of *E. coli* MG1655-A1 was greater than that obtained without the [C₄C₁Im]Cl (Figure 2A). It was noted that the biomass yield of *E. coli* MG1655-A1 in the presence of 250 mM [C₄C₁Im]Cl was greater than those in the presence of 50 and 150 mM [C₄C₁Im]Cl. This suggested that [C₄C₁Im]Cl may stimulate biomass production of *E. coli* MG1655-A1.

The OD₆₀₀ of *E. coli* MG1655-A1 reached 3.5 in the presence of 250 mM [C₄C₁Im]Cl, but *E. coli* MG1655-A1 showed no growth in the presence of 350 mM [C₄C₁Im]Cl, indicating that 350 mM [C₄C₁Im]Cl was lethal for *E. coli* MG1655-A1. Figure 2B shows that the presence of [C₄C₁Im]Cl inhibited growth of *E. coli* MG1655-A2. The OD₆₀₀ reached 1.5 in the presence of 350 mM [C₄C₁Im]Cl and *E. coli* MG1655-A2 has a higher IC₅₀ value than that of *E. coli* MG1655-A1. The parent strain *E. coli* MG1655 could be tolerant only to 50 mM [C₄C₁Im]Cl, reaching a final OD₆₀₀ of 1.2 (Figure 2C). After adaptive evolution, the values of the

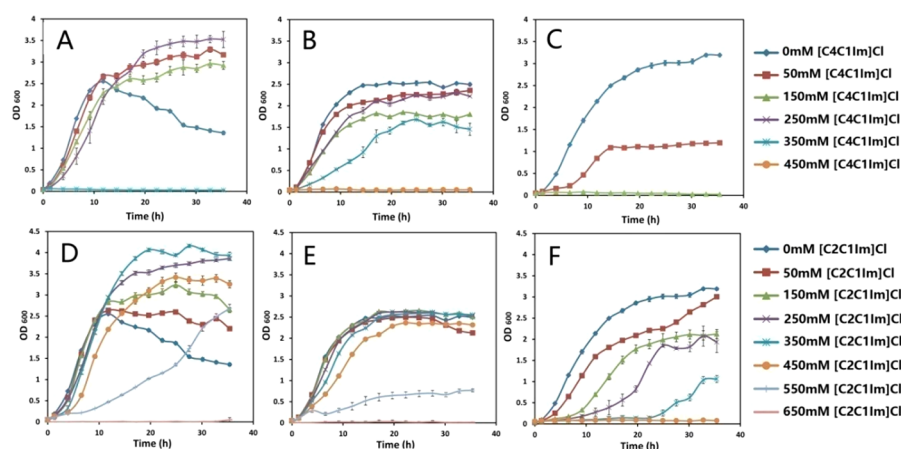


Figure 2. Growth profiles of *E. coli* in the presence of $[C_4C_1Im]Cl$ and $[C_2C_1Im]Cl$. *E. coli* MG1655-A1 (A), *E. coli* MG1655-A2 (B), and *E. coli* MG1655 (C) in the presence of 0, 50, 150, 250, 350, and 450 mM $[C_4C_1Im]Cl$; *E. coli* MG1655-A1 (D), *E. coli* MG1655-A2 (E), and *E. coli* MG1655 (F) in the presence of 0, 50, 150, 250, 350, 450, 550, and 650 mM $[C_2C_1Im]Cl$. ILs were added into M9 medium before fermentation. The error bars represent standard deviation calculated from each experiment run in triplicate.

IC_{50} of *E. coli* MG1655-A1 and *E. coli* MG1655-A2 for $[C_4C_1Im]Cl$ were increased 4.5-fold and 6.1-fold compared to that of the parent strain (Table 2), respectively.

Table 2. IC_{50} (mM) for ILs in Different Microorganisms

| organism | IC_{50} for $[C_4C_1Im]Cl$ | IC_{50} for $[C_4C_1Im][OAc]$ | IC_{50} for $[C_2C_1Im]Cl$ | IC_{50} for $[C_2C_1Im][OAc]$ |
|--------------------------|------------------------------|---------------------------------|------------------------------|---------------------------------|
| <i>E. coli</i> MG1655-A1 | 207 | 142 | 551 | 176 |
| <i>E. coli</i> MG1655-A2 | 321 | 191 | 530 | 286 |
| <i>E. coli</i> MG1655 | 46 | 48 | 225 | 169 |

As shown in Figure 2D, the presence of $[C_2C_1Im]Cl$ increased the biomass yield of *E. coli* MG1655-A1 when the $[C_2C_1Im]Cl$ concentration was in the range of 50–550 mM. The biomass yield decreased with further increase of the $[C_2C_1Im]Cl$ concentration. *E. coli* MG1655-A1 showed no growth in the presence of 650 mM $[C_2C_1Im]Cl$. Interestingly,

the biomass yield increased with the increasing $[C_2C_1Im]Cl$ concentration in the range of 50–350 mM. This shows the stimulation effect of $[C_2C_1Im]Cl$ on the growth of *E. coli* MG1655-A1, similar to that of $[C_4C_1Im]Cl$.

E. coli MG1655-A2 did not show significant differences in growth curves in the presence of 0–450 mM $[C_2C_1Im]Cl$ (Figure 2E), suggesting that this strain was not very sensitive to $[C_2C_1Im]Cl$ within this concentration range. The biomass yield of *E. coli* MG1655-A2 dropped significantly when the $[C_2C_1Im]Cl$ concentration was increased to 550 mM. The IC_{50} value for $[C_2C_1Im]Cl$ of *E. coli* MG1655-A1, *E. coli* MG1655-A2, and *E. coli* MG1655 was 551, 530, and 225 mM, respectively. These results indicated that *E. coli* MG1655-A1's and *E. coli* MG1655-A2's tolerances to $[C_2C_1Im]Cl$ were improved significantly after adaptive evolution. What's more, a certain amount of $[C_2C_1Im]Cl$ or $[C_4C_1Im]Cl$ was shown to simulate the growth of *E. coli* MG1655-A1.

Figure 3 shows growth profiles of the three strains in the presence of $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$. In the presence of low concentrations of ILs (50 and 100 mM for $[C_4C_1Im][OAc]$, 50 mM for $[C_4C_1Im][OAc]$), the biomass yield was greater than that of *E. coli* MG1655-A1 in the absence of the ILs because of the decline phase. This is similar

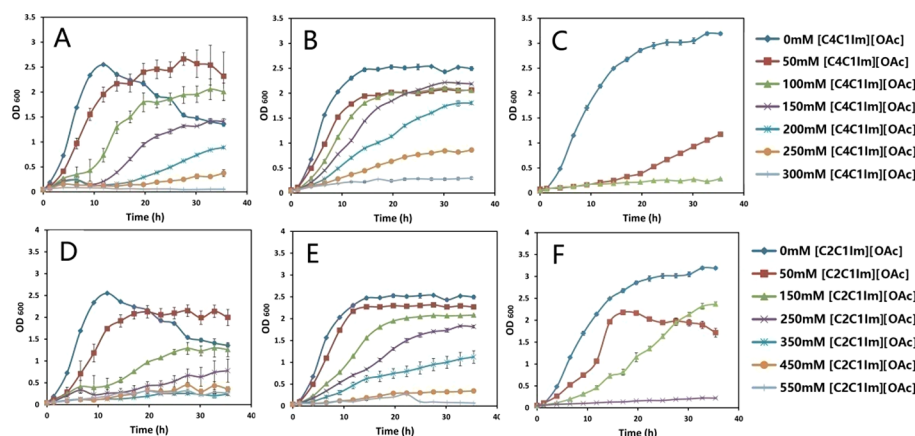


Figure 3. Growth profiles of *E. coli* in the presence of $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$. *E. coli* MG1655-A1 (A), *E. coli* MG1655-A2 (B), and *E. coli* MG1655 (C) in the presence of 0, 50, 100, 150, 200, 250, and 300 mM $[C_4C_1Im][OAc]$; *E. coli* MG1655-A1 (D), *E. coli* MG1655-A2 (E), and *E. coli* MG1655 (F) in the presence of 0, 50, 150, 250, 350, 450, and 550 mM $[C_2C_1Im][OAc]$.

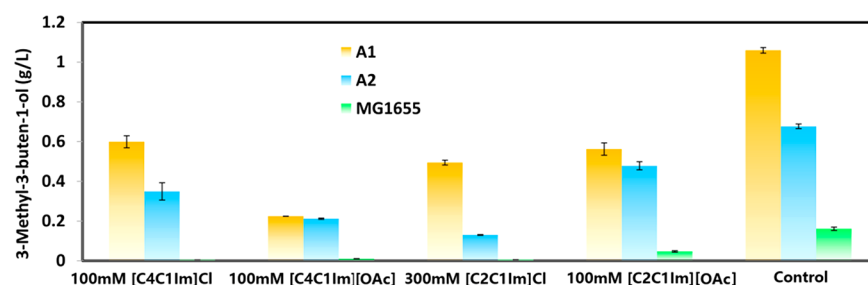


Figure 4. Isoprenol production from *E. coli* MG1655-A1, *E. coli* MG1655-A2, and *E. coli* MG1655 with or without the presence of ILs in EZ-rich medium. The medium of control groups contains no IL. The error bars represent standard deviation calculated from each experiment run in triplicate.

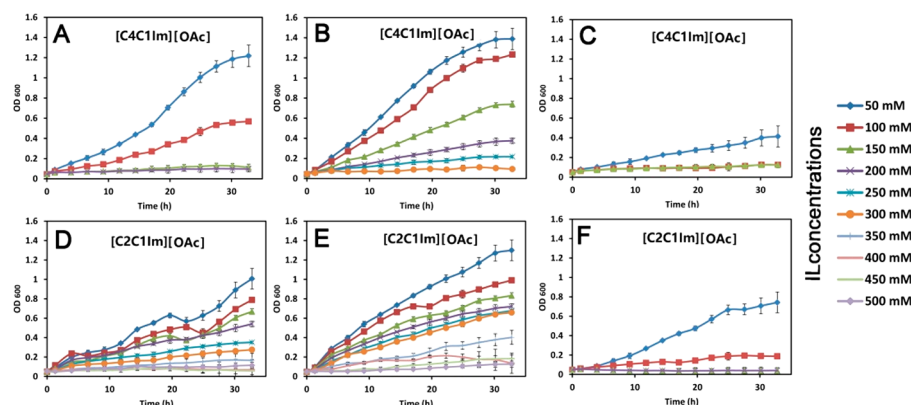


Figure 5. Growth profiles of *E. coli* using ILs as carbon sources. (A) *E. coli* MG1655-A1 treated by $[C_4C_1Im][OAc]$; (B) *E. coli* MG1655-A2 treated by $[C_4C_1Im][OAc]$; (C) *E. coli* MG1655 treated by $[C_4C_1Im][OAc]$; (D) *E. coli* MG1655-A1 treated by $[C_2C_1Im][OAc]$; (E) *E. coli* MG1655-A2 treated by $[C_2C_1Im][OAc]$; (F) *E. coli* MG1655 treated by $[C_2C_1Im][OAc]$. The error bars represent standard deviation calculated from each experiment run in triplicate.

to the cases of $[C_4C_1Im]Cl$ and $[C_2C_1Im]Cl$. With increasing of the concentration of $[C_4C_1Im][OAc]$ or $[C_2C_1Im][OAc]$, the final biomass yield decreased for all three strains. This is different from the growth profiles of the *E. coli* MG1655-A1 in the presence of $[C_4C_1Im]Cl$ or $[C_2C_1Im]Cl$ where a certain amount of ILs promoted its growth. The *E. coli* MG1655 could tolerate only 50 mM $[C_4C_1Im][OAc]$ with a final OD_{600} of 1 (Figure 3C). The *E. coli* MG1655-A1's and *E. coli* MG1655-A2's IC_{50} values for $[C_4C_1Im][OAc]$ were increased 3.0-fold and 4.0-fold compared to the parent strain, respectively (Table 2).

Table 2 shows that values of IC_{50} of the three strains for $[C_2C_1Im][OAc]$ and $[C_2C_1Im]Cl$ are larger than those for $[C_4C_1Im][OAc]$ and $[C_4C_1Im]Cl$, respectively. This suggests that the toxicity of the ILs is related to the alkyl chain length where longer chains lead to higher toxicity. This has been reported in the literature for a variety of ILs.³⁴ The results shown here also suggest that the Cl^- ion is less toxic than the $[OAc]^-$ ion. This also has been reported before.³⁴

The significantly increased IL tolerance in adapted strains *E. coli* MG1655-A1 and *E. coli* MG1655-A2 made them promising hosts for biofuel production from IL-pretreated biomass. To further evaluate whether the adapted strains could improve biofuel production in the presence of ILs, we transformed mevalonate-based metabolic pathway of isoprenol into *E. coli* MG1655-A1, *E. coli* MG1655-A2, and *E. coli* MG1655.³² The metabolic pathway of isoprenol consists of two plasmids: one plasmid contains five mevalonate pathway genes and the other plasmid contains two isoprenol synthase genes. In the EZ-rich medium without ILs, the isoprenol titer of *E. coli* MG1655-A1-

KG1R10, *E. coli* MG1655-A2-KG1R, and *E. coli* MG1655 reached 1.06, 0.68, and 0.16 g/L (Figure 4), respectively. Compared to that in *E. coli* MG1655, the isoprenol titer of *E. coli* MG1655-A1-KG1R10 and *E. coli* MG1655-A2-KG1R10 was increased 6.6-fold and 4.3-fold, respectively. This result indicated that ALE increased not only *E. coli* MG1655's IL tolerance but also *E. coli* MG1655's isoprenol production without the presence of ILs. The increased isoprenol production in tolerant strains could be due to the spontaneous mutations which were beneficial to the metabolic pathway of isoprenol.

Next, 100 mM $[C_4C_1Im]Cl$, 100 mM $[BMIM]Ac$, 300 mM $[C_2C_1Im]Cl$, and 100 mM $[C_2C_1Im][OAc]$ were added in EZ-rich medium (containing 10 g/L glucose) to simulate residual ILs in hydrolysate after IL pretreatment and saccharification.¹⁶ In the presence of 100 mM $[C_4C_1Im]Cl$, 100 mM $[BMIM]Ac$, 300 mM $[C_2C_1Im]Cl$, and 100 mM $[EMIM]Ac$, the isoprenol titer of the *E. coli* MG1655 was lower than 0.1 g/L (Figure 4). However, the *E. coli* MG1655-A1-KG1R10 and *E. coli* MG1655-A2-KG1R10 showed significantly increased isoprenol titers compared to parent strain *E. coli* MG1655 (Figure 4). Furthermore, *E. coli* MG1655-A1-KG1R10s performance in isoprenol production was better than *E. coli* MG1655-A2-KG1R10. The *E. coli* MG1655-A1-KG1R10s isoprenol titer in the presence of 100 mM $[C_4C_1Im]Cl$, 100 mM $[BMIM]Ac$, 300 mM $[C_2C_1Im]Cl$, and 100 mM $[C_2C_1Im][OAc]$ reached 0.60, 0.22, 0.49, and 0.56 g/L, respectively. These results indicated that ALE provided promising host strains for one-pot biofuel production integrating IL pretreatment with saccharification and fermentation.

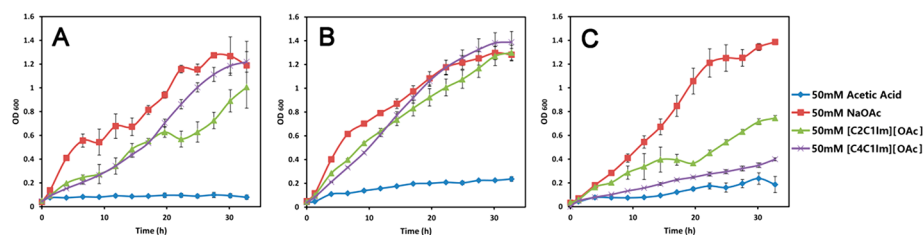


Figure 6. Growth profiles of *E. coli* MG1655-A1 (A), *E. coli* MG1655-A2 (B), and *E. coli* MG1655 (C) using $[C_4C_1Im][OAc]$, $[C_2C_1Im][OAc]$, NaOAc, and acetic acid as carbon sources in M9 medium. The error bars represent standard deviation calculated from each experiment run in triplicate.

$[C_4C_1Im][OAc]$ or $[C_2C_1Im][OAc]$ was used as the sole carbon source for growth and isoprenol fermentation of adapted *E. coli*. During IL-based pretreatments, multiple washing processes were needed to precipitate cellulose^{35,36} and wash off ILs from biomass.¹⁶ Because of their high cost,³⁷ resistance to biodegradation³⁸ and toxicity to microbes³⁹ of certain ILs, they have to be recycled and reused. The ILs containing a certain amount of water need be regenerated by rotary evaporation,^{40,41} which was a commonly used approach. However, it is difficult to use rotary evaporation to recycle the ILs of low concentration; the ILs remained in fermentation liquor of one-pot biofuel production²² and the ILs in other complex industrial wastewater. Therefore, it is necessary to develop an approach to reuse these ILs in wastewater.

Thus, we studied the feasibility of using $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as carbon sources for microbe growth and biofuel fermentation. Figure 5 presents the growth curve of *E. coli* MG1655-A1, *E. coli* MG1655-A2, and *E. coli* MG1655 using $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as carbon sources in M9 medium. When 50 mM $[C_4C_1Im][OAc]$ and 50 mM $[C_2C_1Im][OAc]$ are used as carbon sources, *E. coli* MG1655's OD₆₀₀ reached 0.41 and 0.74, respectively. When the IL concentration was greater than 50 mM, *E. coli* MG1655 could not grow. The adapted strains *E. coli* MG1655-A1 and *E. coli* MG1655-A2 showed better growth performance with $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as carbon sources in M9 medium compared to *E. coli* MG1655. The maximum OD₆₀₀ of *E. coli* MG1655-A1 and *E. coli* MG1655-A2 using 50 mM $[C_4C_1Im][OAc]$ as carbon source reached 1.2 and 1.4, respectively. The maximum OD₆₀₀ of *E. coli* MG1655-A1 and *E. coli* MG1655-A2 using 50 mM $[C_2C_1Im][OAc]$ as the carbon source reached 1 and 1.2, respectively. When using 100 mM $[C_4C_1Im][OAc]$ and 100 mM $[C_2C_1Im][OAc]$ as carbon sources, the OD₆₀₀ of *E. coli* MG1655-A2 could even reach 1.2 and 1.0, respectively. These results suggested that *E. coli* MG1655-A1 and *E. coli* MG1655-A2 could potentially reuse wastewater containing $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as the carbon source for microbe growth. High-performance liquid chromatography (HPLC) analysis (by comparing peak area in the chromatogram) showed that $[C_4C_1Im]^+$ or $[C_2C_1Im]^+$ could not be assimilated by *E. coli* MG1655-A1, *E. coli* MG1655-A2, or *E. coli* MG1655 because the imidazolium ring was resistant to biodegradation.³⁸ These results suggested that the acetate ion in ILs was used as the carbon source. Singer et al. also found that *Aspergillus fumigatus* and *Aspergillus ustus* isolated from lignocellulose-deconstructing environments could use acetate ion in $[C_2C_1Im][OAc]$ as the carbon source.⁴² During the fermentation process, $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ were transformed into $[C_4C_1Im]X$ and $[C_2C_1Im]X$ (X represents various anions), respectively. $[C_4C_1Im]X$ and

$[C_2C_1Im]X$ left in wastewater could be further regenerated by bipolar membrane electrodialysis into hydroxide-based precursors for IL syntheses.^{43,44}

In this study, we also compared the growth behavior of the three strains using the 50 mM $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as carbon sources to that using 50 mM sodium acetate (NaOAc) and acetic acid as carbon sources. The biomass yields of adapted strains using $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as carbon sources did not show significant differences compared to that using NaOAc as the carbon source (Figure 6A,B), even though $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ slightly prolonged lag phase. However, for *E. coli* MG1655, using $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as carbon sources caused a significant inhibition to growth compared to that using NaOAc as the carbon source (Figure 6C). The biomass yields of *E. coli* MG1655 using $[C_4C_1Im][OAc]$, $[C_2C_1Im][OAc]$, and NaOAc as carbon sources were in the following order: NaOAc > $[C_2C_1Im][OAc]$ > $[C_4C_1Im][OAc]$. For both *E. coli* MG1655 and adapted *E. coli*, acetic acid was most toxic among the four tested carbon sources. These results indicated that within a certain range the toxicity of $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as the carbon source for adapted strains was similar to that of NaOAc, which could be used as the carbon source for lipid production.⁴⁵

To further evaluate whether $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ could be used as carbon sources for biofuel production, we used 50 mM $[C_4C_1Im][OAc]$ and 50 mM $[C_2C_1Im][OAc]$ as carbon sources to produce isoprenol. The parent strains *E. coli* MG1655-KG1R10s isoprenol titer using 50 mM $[C_4C_1Im][OAc]$, 50 mM $[C_2C_1Im][OAc]$, and 50 mM NaOAc as carbon source was lower than 14 mg/L (Figure 7). However, the strains *E. coli* MG1655-A1-KG1R10 and *E. coli* MG1655-A2-KG1R10 showed significantly increased isoprenol titer using the ILs or NaOAc as carbon sources

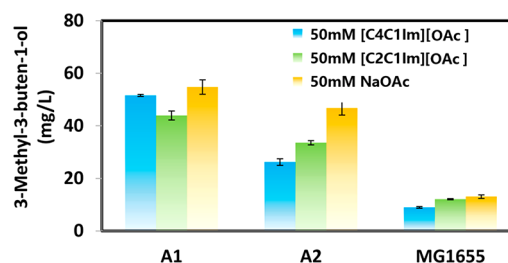


Figure 7. Isoprenol production of *E. coli* MG1655-A1-KG1R10, *E. coli* MG1655-A2-KG1R10, and *E. coli* MG1655-KG1R10 using $[C_4C_1Im][OAc]$, $[C_2C_1Im][OAc]$, and NaOAc as carbon source in EZ-rich medium. The error bars represent standard deviation calculated from each experiment run in triplicate.

(Figure 7). Furthermore, *E. coli* MG1655-A1-KG1R10's performance in isoprenol production was better than *E. coli* MG1655-A2-KG1R10's (Figure 7). *E. coli* MG1655-A1-KG1R10s isoprenol titer using 50 mM $[C_4C_1Im][OAc]$ and 50 mM $[C_2C_1Im][OAc]$ as carbon source reached 52 and 44 mg/L, respectively. *t* test showed that the isoprenol titer using $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as carbon sources in *E. coli* MG1655-A1-KG1R10 did not show significant differences compared to that using NaOAc as the carbon source ($p > 0.05$), indicating that *E. coli* MG1655-A1-KG1R10 was a promising host to reuse acetate of $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ in wastewater for biofuel production.

CONCLUSIONS

In this study, we characterized the growth and biofuel production of IL-tolerant *E. coli* obtained by adaptive laboratory evolution. Adapted *E. coli* showed increased biomass yield, increased IC_{50} , and increased biofuel production compared to the parent strain. The growth of *E. coli* MG1655-A1 was even promoted by $[C_4C_1Im]Cl$ and $[C_2C_1Im]Cl$ of certain concentrations. The adapted *E. coli* provided new host strains for one-pot biofuel production integrating IL-based pretreatment with saccharification and fermentation. We also found that *E. coli* MG1655-A1 was a promising host for using the acetate ion of $[C_4C_1Im][OAc]$ - and $[C_2C_1Im][OAc]$ in wastewater for biofuel production, providing an approach to reuse and recycle ILs in wastewater.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.8b05144.

Bacterial strains, medium and culture conditions, characterization of adapted *E. coli*'s IL tolerance, growth assay using $[C_4C_1Im][OAc]$ and $[C_2C_1Im][OAc]$ as carbon sources, biodegradation assay, isoprenol fermentation, and production assay (PDF)

AUTHOR INFORMATION

Corresponding Authors

*E-mail: SWSinger@lbl.gov. Mailing address: Deconstruction Division, Joint BioEnergy Institute, 5885 Hollis Street, Emeryville, CA 94608, USA. (S.W.S.).

*E-mail: chenggang@mail.buct.edu.cn. Mailing address: Science and Technology Building, West Wing 404, Beijing University of Chemical Technology, North 3rd Ring East, #15, Beijing 100029, China. (G.C.)

ORCID

Gang Cheng: 0000-0003-2014-7838

Notes

The authors declare no competing financial interest.

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